NOTES

Forest Science, Vol. 33, No. 2, pp. 548–551.

Rates of Free-Living Nitrogen Fixation in Some Piedmont Forest Types

Deborah Grant and Dan Binkley

ABSTRACT. We examined rates of free-living (nonsymbiotic) nitrogen fixation in a successional sequence of forest types in the Piedmont of North Carolina. Annual nitrogen input through this process did not differ among ecosystems, averaging roughly 0.002 kg/ha annually. The rate in a 20- to 30-year-old stand did not increase with irrigation or additions of nitrogen, phosphorus, or molybdenum. Free-living nitrogen fixation in these forests should not be expected to compensate for nitrogen losses through natural processes or management activities. FOR. SCI. 33(2):548–551.
ADDITIONAL KEY WORDS. Acetylene reduction, loblolly pine, nitrogen cycling.

THE AVAILABILITY OF NITROGEN (N) limits productivity in many forests, and management activities such as prescribed fire, harvest, and site preparation can decrease the N content of an ecosystem. Annual inputs of N from precipitation and N fixation may replace these losses over the period of one or more rotations. Across the range of loblolly pine (*Pinus taeda* L.), N inputs in precipitation probably average about 6 kg/ha annually (Jorgensen and Wells 1986). Inputs from N fixation in this area are poorly quantified; high rates of 10 kg/ha annually may occur in the presence of symbiotic N-fixers such as wax myrtle (*Myrica cerifera* L.) (Permar and Fisher 1983). Little information is available on rates of free-living (nonsymbiotic) N fixation in the pine forests in the Southeastern United States, but one study reported rates of less than 1 kg/ha annually (Jorgensen and Wells 1971). Our objective in the present study was to supplement previous work by estimating the magnitude of free-living N fixation in a successional sequence of stands in the Duke Forest in the North Carolina Piedmont, and to test some factors that might limit the rate.

Site Description

The four study sites were chosen to represent common stages of forest succession in the region: a 1-year-old clearcut planted with loblolly pine, a 20- to 30-year-old old-field loblolly pine stand, an 80- to 90-year-old stand of loblolly pine with some Virginia pine (*Pinus echinata* Mill.), and an old, uneven-aged hardwood stand dominated by red oak (*Quercus rubra* L.), white oak (*Q. alba* L.), and black oak (*Q. velutina* Lam.). All stands were located on Georgeville or Herndon soil series; these series differ only slightly in horizon development, and are both clayey, kaolinitic, thermic Typic Hapludults. The pH of the soil of all sites fell within the range of 5 to 6. Soils, nutrient cycles, and productivity of these sites were characterized by Saterson (1985). Annual precipitation averages 115 cm, with about 60 cm falling from April to September. During our sampling in 1985, precipitation was above normal.

Methods

We incubated samples of the forest floor plus upper 4 cm of mineral soil (collected with a 6-cm diameter core) in a 10% atmosphere of acetylene (C_2H_2) to evaluate N-fixation rates (McNabb and Geist 1979). Five samples were collected at each site,

The authors were Graduate Research Assistant and Assistant Professor of Forest Ecology and Soils, respectively, School of Forestry and Environmental Studies, Duke University. This project was funded by the Duke University Research Council. We thank J. Siedow for use of lab facilities. Address reprint requests to D. Binkley, Dept. of Forest and Wood Sciences, Colorado State University, Ft. Collins, CO 80523. Manuscript received August 27, 1986.

three times weekly, for the months of May, June, July, and November of 1985. Each sample was placed in a 1 l plastic bottle with a serum stopper in the lid. One hundred ml of air was removed with a syringe and replaced with 100 ml of acetylene that was generated by adding distilled water to calcium carbide (CaC_2). The samples were incubated on-site for 24 hours beneath a layer of litter to maintain ambient temperature. Rates of acetylene reduction were so low that incubation periods less than 24 hours did not give reproducible results. After incubation, 5 ml samples of bottle atmosphere were removed, and analyzed for acetylene and ethylene by gas chromatography. Analysis of variance was used to examine difference in means across sites and months.

We examined factors that might limit N-fixation rates in the 20- to 30-year-old pine stand with three replicates per treatment in a completely randomized experiment with additions of water, or all possible combinations of N, phosphorus (P), and molybdenum (Mo). Treatments were applied to 1 m $\times$ 1 m quadrats, with a 0.3 m buffer between quadrats. Water was applied at 5 cm per week for 3 weeks before the assay in June, with the last addition 4 days prior to the assay. Fertilizers were applied 6 weeks before the June assay, at rates of 10 g/m^2 N (as ammonium nitrate), 5 g/m^2 P (as monobasic sodium phosphate), and 25 mg/m^2 Mo (as ammonium molybdate). The quadrats were also sampled in August; 5 cores/quadrat were assayed at both times. Analysis of variance was used to compare means for treatments and sampling times.

Results and Discussion

The average monthly rates of acetylene reduction did not differ among sites, although the rates differed significantly across months (Table 1). May and June rates significantly exceeded the rates for July and November. We also found no significant effect of the treatments applied in the 20- to 30-year-old pine stand, though again the acetylene reduction rate differed by month (Table 2). The highest rates we observed came in the August sampling of the fertilization experiment when the average rate was almost an order of magnitude above those recorded in the 4 months of sampling in the untreated stands.

Given the high variability within and among sites and times, and uncertainty in the conversion ratio for acetylene reduction to N_2 reduction, it is not possible to calculate a precise estimate of free-living N fixation for these stands. A very rough approximation can be calculated by multiplying the across-site and across-month average rate in Table 1 of 1.7 μmol C_2H_2 reduced per m^2 monthly by 12 months, by 10,000 m^2/ha, and by ⅓ mol N_2 fixed per mol C_2H_2 reduced. This gives 68 mmol of N_2 fixed per ha annually, or 1.9 g N/ha annually. Some assumptions behind these calculations may be in error, but the values would be vey low even if our estimate were an order of magnitude below the real rate. Despite possible sources of uncertainty, free-living N fixation appears negligible in these forests relative to atmospheric inputs and other N cycle processes.

Most other studies in the region have found very low rates of free-living N fixation, with the apparent exception of very wet sites. Jorgensen and Wells (1971) re-

TABLE 1. Acetylene reduction rates ($\mu mol\ C_2H_2\ m^{-2}\ month^{-1}$) in untreated stands by sampling time and site (mean and standard deviation). Rates differed significantly by month ($P = 0.03$), but site and site-by-month interaction were not significant ($P > 0.10$).

	Month				
Site	May	June	July	November	Average
1-yr clearcut	3.6 (6.3)	2.9 (3.4)	0.3 (0.4)	0.6 (0.6)	1.9
20–30-yr pine	2.2 (4.6)	4.9 (5.2)	1.5 (1.3)	1.2 (1.2)	2.5
80–90-yr pine	0.5 (0.9)	3.3 (4.0)	0.2 (0.2)	0.3 (0.3)	1.1
Uneven-age hardwood	1.6 (1.7)	2.6 (2.1)	0.4 (0.2)	0.6 (0.6)	1.3
All-sites	2.0	3.4	0.6	0.6	1.7

TABLE 2. Acetylene reduction rates (μmol C_2H_2 m^{-2} $month^{-1}$) after irrigation and nutrient additions in the 20- to 30-year-old pine stand. Rates differed by month ($P <$ 0.001), but not by treatment.

Treatment	June	August
Control	2.5	18.6
Water	3.0	16.7
+ Mo	2.0	22.5
+ P	2.0	19.2
+ N	9.3	14.3
+ Mo + P	2.3	21.1
+ Mo + N	5.6	23.7
+ P + N	6.3	16.6
+ Mo + P + N	2.5	17.0
Average	3.9	18.8

ported annual free-living N fixation rates in poorly to very poorly drained loblolly pine stands in the Coastal Plain of South Carolina of about 45 g/ha for unburned sites and 950 g/ha for burned sites. Later work by Jorgensen (1975) also found low rates for upland pine sites, but estimates for some swamps approached 10,000 g N/ha annually. Vance et al. (1983) estimated the rate in an oak-hickory forest in Missouri to be only 100 g N/ha annually, in sharp contrast to the 9,200 g N/ha annual rate reported by Todd et al. (1978) for a similar forest in North Carolina. The higher rate in North Carolina could relate to higher rainfall (as suggested by Vance et al. 1983), but it is difficult to evaluate the North Carolina study since no sampling methodology was included in the report. Di Stefano (1984) estimated annual rates of 2,000 to 3,000 g/ha for some very poorly drained, very acidic soils from slash pine (*Pinus elliotti* Engelm.) stands in northern Florida. He found that fertilization and burning produced mixed effects across sites and seasons with statistically strong interactions.

It is risky to generalize from a base of so few studies, each using different methods on different sites. However, it appears that free-living N fixation rates in Southeastern forests are negligible with the possible exception of very wet sites. Our irrigation treatment indicated that free-living N fixation was not limited by water availability. Therefore, higher rates in the very poorly drained sites probably relate more to low oxygen tensions that enhance N fixation rates (Fay 1981, Jensen 1981) than to an adequate supply of water. The quantities of N added to most upland forests in this region through free-living N fixation are probably less than the uncertainty involved in estimates of precipitation inputs. The rates may be significant on very poorly drained sites, however, and more research is needed to test this pattern. At this point, forest managers should not expect free-living N fixation to make substantial contributions to the N capital of most forests in this region.

Literature Cited

DI STEFANO, J. 1984. Nitrogen mineralization and non-symbiotic nitrogen fixation in an age sequence of slash pine plantations in North Florida. Doctoral diss., University of Florida, Gainesville. 219 p.

FAY, P. 1981. Photosynthetic micro-organisms. P. 1–29 *in* W. J. Broughton (ed.), Nitrogen fixation, Volume 1, ecology. Clarendon, Oxford.

JENSEN, V. 1981. Heterotrophic micro-organisms. P. 30–56 *in* W. J. Broughton (ed.), Nitrogen fixation, Volume 1, ecology. Clarendon, Oxford.

JORGENSEN, J. R. 1975. Nitrogen fixation in forested coastal plain soils. USDA For. Serv. Res. Pap. SE-130.

JORGENSEN, J. R., and C. G. WELLS. 1971. Apparent nitrogen fixation in soil influenced by prescribed burning. Soil. Sci. Soc. Am. Proc. 35:806–810.

JORGENSEN, J. R., and C. G. WELLS. 1986. Foresters' primer in nutrient cycling: a loblolly

pine management guide. USDA For. Serv. Southeast. For. Exp. Stn. Gen. Tech. Rep. SE-37. 42 p.

McNabb, D., and J. M. Geist. 1979. Acetylene reduction assay of symbiotic N_2 fixation under field conditions. Ecology 1070–1072.

Permar, T. A., and R. F. Fisher. 1983. Nitrogen fixation and accretion by wax myrtle (*Myrica cerifera*) in slash pine (*Pinus elliottii*) plantations. For. Ecol. Manage. 5:39–46.

Saterson, K. A. 1985. Nitrogen availability, primary production, and nutrient cycling during secondary succession in North Carolina Piedmont forests. Doctoral diss., University of North Carolina, Chapel Hill. 212 p.

Todd, R. L., R. D. Meyer, and J. B. Waide. 1978. Nitrogen fixation in a deciduous forest in the Southeastern United States. *In* U. Granhall (ed.), Environmental role of nitrogen-fixing blue-green algae and symbiotic bacteria. Ecol. Bull. 26:172–177.

Vance, E. D., G. S. Henderson, and D. G. Blevins. 1983. Nonsymbiotic nitrogen fixation in an oak-hickory forest following long-term prescribed burning. Soil Sci. Soc. Am. J. 47:134–147.